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Active Sequences:

SEQ ID NO:1 (MW=7670)
AELRCMCIKTTSGIHPKNIQSLEVIGKGTHCNQVEVIATLKDGRKICLDPDAPRIKKIVQKKLAGDESAD

SEQ ID NO:2 (MW=8218)
DSDLYAELRCMCIKTTSGIHPKNIQSLEVIGKGTHCNQVEVIATLKDGRKICLDPDAPRIKKIVQKKLAGDESAD

SEQ ID NO:11 (MW=2850)
DSDLYAELRCMCIKTTSGIHPKNIQS

SEQ ID NO:12 (MW=1530)
IKTTSGIHPKNIES

SEQ ID NO:13 (MW=1862)
CMCIKTTSGIHPKNIQS

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SEQ ID NO:14 (MW=1750)
MCIKTTSGIHPKNIQS

SEQ ID NO:15 (MW=1643)
CIKTTSGIHPKNIQS

(57) Abstract

Polypeptides which increase or promote mammalian bone growth, related nucleotide sequences, antibodies, diagnostic kits and treatments. Subsequences of the polypeptide Asp Ser Asp Leu Tyr Ala Glu Leu Arg Cys Met Cys Ile Lys Thr Thr Ser Gly Ile His Pro Lys Asn Ile Gln Ser Leu Glu Val Ile Gly Lys Gly Thr His Cys Asn Gln Val Glu Val Ile Ala Thr Leu Lys Asp Gly Arg Lys Ile Cys Leu Asp Pro Asp Ala Pro Arg Ile Lys Lys Ile Val Gln Lys Lys Leu Ala Gly Asp Glu Ser Ala Asp have been shown to promote growth. Subsequences include Asp Ser Asp Leu Tyr Ala Glu Leu Arg Cys Met Cys Ile Lys Thr Thr Ser Gly Ile His Pro Lys Asn Ile Gln Ser; Ile Lys Thr Thr Ser Gly Ile His Pro Lys Asn Ile Glu Ser; and Cys Met Cys Ile Lys Thr Thr Ser Gly Ile His Pro Lys Asn Ile Gln.

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BONE STIMULATING FACTOR

The present invention relates to polypeptides which stimulate bone growth.

Understanding of issues related to bone growth and strength has progressed over the years, a summary being provided in, for example, international patent application No. PCT/CA 94/00144, published on September 15, 1994 under WO 94/20615, United States Patent No. 5,320,970 and European patent application No. 92 302 446, published under 505 210 on September 23, 1992, the contents of which applications are incorporated herein by reference.

By way of background to the present invention, described below, neutrophil-activating peptide (NAP-2; SEQ ID NO:1) and a variant of NAP-2, here termed "NAP-2V" (SEQ ID NO:2) have been known for some time (Walz, A., and M. Baggiolini, (1989) *Biochem. Biophys. Res. Commun.* **159**:969). British Patent No. 2 231 872 (British Patent No. 2 231 872. Inventors: M. Baggiolini, K.J. Clemetson, and A. Walz. Published June 14, 1990.), describes the amino acid sequence of NAP-2 and three apparently naturally occurring variants, including NAP-2V. The other two variants have an additional four (SEQ ID NO:3) and three (SEQ ID NO:4) amino acids at the N-terminal of the NAP-2 sequence. NAP-2 is a subsequence of ß-thromboglobulin (ß-TG; SEQ ID NO:5) which has an additional eleven amino acids at the N-terminal end. ß-TG is itself a subsequence of connective tissue-activating peptide (CTAP-III; SEQ ID NO:6) which has an additional four amino acids at the N-terminal. CTAP-III is a subsequence of platelet basic protein (PBP; SEQ ID NO:7) which has an additional nine amino acids at the N-terminal.

NAP-2 along with interleukin-8 (human IL-8; SEQ ID NO:8; porcine IL-8 SEQ ID NO:9) and melanoma growth-stimulating activity (MGSA) have been assigned to a subfamily known as the α-chemokines. The α-chemokines have in common with each other four cysteine residues at highly conserved positions, which enclose the core region of the molecules, as described by Brandt *et al* (Ehlert, J.E., F. Peterson, M.H.G. Kubbuta, J. Gerdes, H.-D. Flad, and E. Brandt, (1995) *J. Biol. Chem.* **270**:6338). Brandt *et al.* found an apparently naturally occurring C-terminus truncated variant of NAP-2, lacking the last four amino acids of NAP-2, that displays enhanced increase in potency to stimulate neutrophil degranulation. Brandt *et al.* also synthesized variants lacking the final one, two, three, five and six amino acids of the C-terminus of NAP-2. All of these C-truncated polypeptides exhibited a moderate increase in potency over NAP-2 with the exception of the sequence having only the first sixty-four amino acids of NAP-2. Brandt *et al.* discussed the possible significance of the sequence modifications with respect to the structure of NAP-2 and its function.

Platelet factor 4 (PF4; SEQ ID NO:10) is a seventy amino acid polypeptide (Hermodson, M., G. Schmer and K. Kurachi, (1977) *J. Biol. Chem.* 252:6276; Morgan, F.J., G.S Begg, C.N. Chesterman, (1979) *Thromb. Haemost.* **42**:1652). PF4 has been shown to inhibit proliferation of two osteoblastic osteosarcoma cell lines, Saos-2 and G-292 (United

States Patent No. 5,304,542. Inventor: D.M. Tatakis. Issued April 19, 1994). Indomethacin apparently did not affect PF4-induced inhibition of the cell proliferation. Particular fragments, PF4(58-70), PF4(47-70) and monomeric low-affinity PF4 (LAPF4), which is 50% homologous to PF4 and contains an α- helical C-terminus were also suggested as being useful. PF4 and such related polypeptides were thus described as being useful in a method for inhibiting proliferation of osteoblasts, in among other things, humans suffering from osteoporosis.

The first 70 amino acids of NAP-2V and the sequence of PF4 are about 51% homologous and the positions of the four cysteine residues are conserved between the two polypeptides.

It has now been shown that NAP-2, NAP-2V, as well as subsequences of NAP-2V also show bone stimulatory effects, while certain subsequences do not display bone stimulatory activity. NAP-2V-(1-26) (SEQ ID NO:11) and NAP-2V-(13-26; gln^{25}-glu^{25}) (SEQ ID NO:12) were found to increase the observed bone apposition rate, the latter of the two being more potent than the former. NAP-2V-(10-26) (SEQ ID NO:13) appeared to cause a small increase in the observed bone apposition rate, although the statistical significance of the observed increase was questionable. NAP-2V-(11-26) (SEQ ID NO:14) and NAP-2V-(12-26) (SEQ ID NO:15) were found to have no effect on observed bone mineral apposition rate.

The invention thus includes a polypeptide which promotes bone growth in mammals, where the polypeptide includes an amino acid sequence corresponding to SEQ ID NO:2 with (a) from 6 to about 12 amino acids deleted from the N-terminus of SEQ ID NO:2, (b) 7 to about 49 amino acids deleted from the C-terminus of SEQ ID NO:2, or both (a) and (b); wherein the sequence includes no cysteine residues or at least two cysteine residues; or a functionally equivalent homologue.

In another aspect, a polypeptide of the present invention is an amino acid sequence corresponding to SEQ ID NO:11 up to 69 amino acids in length, or corresponding to SEQ ID NO:11 with from 6 to about 12 amino acids deleted from the N-terminus of SEQ ID NO:11, or corresponding to SEQ ID NO:11 with from 6 to about 9 amino acids deleted from the N-terminus of SEQ ID NO:11; wherein the sequence includes no cysteine residues or at least two cysteine residues; or a functionally equivalent homologue.

Alternatively, the invention is a polypeptide which promotes bone growth in mammals, where the polypeptide has an amino acid sequence corresponding to SEQ ID NO:12 up to 69 amino acids in length; or a functionally equivalent homologue; or having an amino acid sequence consisting essentially of the amino acid sequence corresponding to SEQ ID NO:11; or a conservatively substituted variant thereof; or a polypeptide having an amino acid sequence consisting essentially of the amino acid sequence corresponding to SEQ ID NO:12; or a conservatively substituted variant thereof; or a polypeptide having an amino acid

sequence consisting essentially of the amino acid sequence corresponding to SEQ ID NO:13; or a conservatively substituted variant thereof.

A polypeptide of the present invention can have at least two cysteine residues which are located at positions corresponding to the tenth and twelfth positions of SEQ ID NO:2.

A polypeptide of the present invention can have, if desired, or necessary, one or the other or both of the N-terminal amino acid and the C-terminal amino acid protected by a protecting group.

The polypeptide can be synthetic and the amino acid sequence can have a molecular weight in the range of from about 1000 to 4000; or from about 1500 to about 3000; or more preferably from about 1500 to about 1800.

In another aspect, the invention is a first polypeptide comprising a sequence of amino acids sufficiently duplicative of a second polypeptide having an amino acid sequence corresponding to SEQ ID NO:2 with (a) from 6 to about 12 amino acids deleted from the N-terminus of SEQ ID NO:2, (b) 7 to about 49 amino acids deleted from the C-terminus of SEQ ID NO:2, or both (a) and (b); wherein the sequence includes no cysteine residues or at least two cysteine residues; or a functionally equivalent homologue, such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide. The DNA sequence of NAP-2V disclosed by Walz *et al.* (British Patent No. 2 231 872. Inventors: M. Baggiolini, K.J. Clemetson, and A. Walz. Published June 14, 1990. Neutrophil-activating peptide-2 and processes for the production of NAP-2, B-TG, CTAP-III and PBP) is identified here as SEQ ID NO:16.

It will, of course, be understood by those skilled in the art that portions of the nucleic acid sequence identified as SEQ ID NO:16 correspond to sequences coding for the polypeptides identified as SEQ ID NO:1, NO:11; NO:12 (Glu-Gln) or NO:13. For example, nucleic acids 37 through 78 of SEQ ID NO:16 encode the amino acid subsequence identified as SEQ ID NO:12 in which the penultimate amino acid of the subsequence is glutamine.

"Stringent hybridization conditions" takes on its common meaning to a person skilled in the art here. Appropriate stringency conditions which promote nucleic acid hybridization, for example, 6x sodium chloride/sodium citrate (SSC) at about 45°C are known to those skilled in the art. The following examples are found in Current Protocols in Molecular Biology, John Wiley & Sons, NY (1989), 6.3.1-6.3.6: For 50 ml of a first suitable hybridization solution, mix together 24 ml formamide, 12 ml 20x SSC, 0.5 ml 2 M Tris-HCl pH 7.6, 0.5 ml 100x Denhardt's solution, 2.5 ml deionized H_2O, 10 ml 50% dextran sulfate, and 0.5 ml 10% SDS. A second suitable hybridization solution can be 1% crystalline BSA (fraction V), 1 mM EDTA, 0.5 M Na_2HPO_4 pH 7.2, 7% SDS. The salt concentration in the wash step can be selected from a low stringency of about 2x SSC at 50°C to a high stringency of about 0.2x

SSC at 50°C. Both of these wash solutions may contain 0.1% SDS. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C to high stringency conditions, at about 65°C. The cited reference gives more detail, but appropriate wash stringency depends on degree of homology and length of probe. If homology is 100%, a high temperature (65°C to 75°C) may be used. If homology is low, lower wash temperatures must be used. However, if the probe is very short (<100bp), lower temperatures must be used even with 100% homology. In general, one starts washing at low temperatures (37°C to 40°C), and raises the temperature by 3-5°C intervals until background is low enough not to be a major factor in autoradiography.

Alternatively, such a first polypeptide is a sequence of amino acids sufficiently duplicative of a second polypeptide having an amino acid sequence corresponding to SEQ ID NO:11 up to 69 amino acids in length, or corresponding to SEQ ID NO:11 with from 6 to about 12 amino acids deleted from the N-terminus of SEQ ID NO:11, or corresponding to SEQ ID NO:11 with from 6 to about 9 amino acids deleted from the N-terminus of SEQ ID NO:11; wherein the sequence includes no cysteine residues or at least two cysteine residues; or a functionally equivalent homologue, such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide.

The first polypeptide can have a sequence of amino acids sufficiently duplicative of a second polypeptide having an amino acid sequence corresponding to SEQ ID NO:12 up to 69 amino acids in length; or a functionally equivalent homologue, such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide.

The first polypeptide can have a sequence of amino acids sufficiently duplicative of a second polypeptide having an amino acid sequence corresponding to to SEQ ID NO:11; or a conservatively substituted variant thereof, such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide; or it can have a sequence of amino acids sufficiently duplicative of a second polypeptide comprising an amino acid sequence corresponding to SEQ ID NO:12; or a conservatively substituted variant thereof, such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide; or it can have a sequence of amino acids sufficiently duplicative of a second polypeptide having an amino acid sequence corresponding to SEQ ID NO:13; or a conservatively substituted variant thereof, such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide.

A bone growth polypeptide of the invention can be of any suitable length, and particularly can be up 14amino acids in length, or up to 20 amino acids in length, or up to 30

amino acids in length, or up to 40 amino acids in length, or up to 50 amino acids in length, or up to 60 amino acids or more in length.

The invention includes any number of chimeric bone stimulating factors made having an amino acid sequence of polypeptides of the present invention.

In another aspect, the invention is an agent for use in prevention and treatment of a bone reduction related disease which includes any polypeptide or polypeptides of the present invention as an active ingredient.

The invention is, alternatively, a pharmaceutical composition for promoting bone growth, having a therapeutically effective amount of a polypeptide or polypeptides of the present invention.

The invention includes a method of increasing bone growth in a mammal by administering a therapeutically effective amount of a polypeptide having an amino acid sequence of a polypeptide or polypeptides of the present invention.

The invention includes use of a polypeptide or polypeptides of the present invention for the treatment of osteoporosis. Alternatively, the use of a polypeptide or polypeptides can be to promote bone growth in a mammal.

The invention includes use of the polypeptide or polypeptides in the preparation of a medicament for use in promoting bone growth or the treatment of osteoporosis.

The invention includes a diagnostic kit for determining the presence of a polypeptide or polypeptides of the present invention. The kit can includes an antibody to a said polypeptide(s) linked to a reporter system wherein the reporter system produces a detectable response when a predetermined amount of the polypeptide(s) and the antibody are bound together.

The invention includes an antibody synthesized using a polypeptide consisting of an amino acid sequence identified as SEQ ID NO:11; SEQ ID NO:12; or SEQ ID NO:13 or a conservatively substituted variant thereof.

More generally, the invention includes an antibody which binds to a polypeptide or polypeptides of the present invention, synthesized using the polypeptide(s).

The invention includes an isolated DNA fragment which encodes the expression of any of the polypeptides of the present invention, and DNA which differs from the fragment due to the degeneracy of the genetic code.

The invention includes a vector comprising a DNA sequence which encodes the expression of any of any of the polypeptides of the present invention.

The invention includes a process for producing a polypeptide of the invention which includes the steps of:

a) preparing a DNA fragment containing a nucleotide sequence which encodes the polypeptide;

b) incorporating said DNA fragment into an expression vector to obtain a recombinant DNA fragment which contains the DNA fragment and is capable of undergoing replication;

c) transforming a host cell with the recombinant DNA fragment to isolate a transformant which can express the polypeptide; and

d) culturing the transformant to allow the transformant to produce the polypeptide and recovering the polypeptide from resulting cultured mixture.

In yet another aspect, the invention is a synthetic polypeptide up to 65 amino acids in length, having *in vivo* bone stimulatory activity in mammals, having an amino acid sequence which is at least about 19% conserved in relation to the amino acid sequence identified as SEQ ID NO:2; a conservatively substituted variant thereof; or a functionally equivalent homologue.

The synthetic polypeptide can also be at least about 22%, 25%, 28%, 31%, or 35% conserved in relation to the amino acid sequence identified as SEQ ID NO:2; a conservatively substituted variant thereof; or a functionally equivalent homologue.

Such a synthetic polypeptide can have at least 49 amino acids deleted from the sequence.

The polypeptide can have no cysteine residues or at least two cysteine residues.

The polypeptide can have a molecular weight in the range of from about 1000 to 4000.

In another aspect, the present invention is a first polypeptide having a sequence of amino acids sufficiently duplicative of a second polypeptide which includes an amino acid sequence of any of the synthetic polypeptides, such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide.

The invention includes a chimeric bone stimulating factor comprising an amino acid sequence of any of the synthetic polypeptides.

An agent of the present invention for use in prevention and treatment of a bone reduction related disease can include one or more of the synthetic polypeptides.

As well, a pharmaceutical composition of the present invention for promoting bone growth, can include a therapeutically effective amount of a one or more of the synthetic polypeptides.

The invention includes a method of increasing bone growth in a mammal by administering a therapeutically effective amount of one or more of the synthetic polypeptides.

Such a synthetic polypeptide can be used for the treatment of osteoporosis or to promote bone growth in a mammal.

Such a synthetic polypeptide can be used in the preparation of a medicament for use in promoting bone growth or the treatment of osteoporosis.

The invention includes a diagnostic kit for determining the presence of one or more the synthetic polypeptides, the kit including antibody(ies) to a the polypeptide(s) linked to a reporter system wherein the reporter system produces a detectable response when a predetermined amount of the polypeptide(s) and the antibody(ies) are bound together.

The invention includes an antibody which binds to one or more of the synthetic polypeptides, synthesized using the polypeptide.

Further still, the invention includes an isolated DNA fragment which encodes the expression of any of the synthetic polypeptides, and DNA which differs from the fragment due to the degeneracy of the genetic code. The invention includes a vector which includes such a DNA sequence.

The invention includes a process for producing any of the synthetic polypeptides, which includes:

a) preparing a DNA fragment containing a nucleotide sequence which encodes a polypeptide;

b) incorporating said DNA fragment into an expression vector to obtain a recombinant DNA fragment which contains the DNA fragment and is capable of undergoing replication;

c) transforming a host cell with the recombinant DNA fragment to isolate a transformant which can express the polypeptide; and

d) culturing the transformant to allow the transformant to produce the polypeptide and recovering the polypeptide from resulting cultured mixture.

The invention includes a polypeptide exhibiting bone stimulatory activity in mammals, the polypeptide being up to 65 amino acids in length and having the sequence identified as SEQ ID NO:11, SEQ ID NO:12, or SEQ ID NO:13; analogues thereof wherein the amino acids in the sequence may be substituted, deleted or added, so long as the bone stimulatory activity in mammals derived the three dimensional structure of the sequence is preserved; and conjugates of each of the polypeptides or analogues thereof, wherein if the polypeptide sequence contains a cysteine residue, there are at least two cysteine residues. Such a polypeptide can be substantially pure and the amino acid sequence can have a molecular weight in the range of from about 1000 to about 4000, or from about 1500 to about 3000, or from about 1500 to about 1800. The invention is also a first polypeptide that includes a sequence of amino acids sufficiently duplicative of a second polypeptide having an amino acid sequence corresponding such a polypeptide, or a functionally equivalent homologue

thereof, such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide; or a chimeric bone stimulating factor including such an amino acid sequence; or an agent for use in prevention and treatment of a bone reduction related disease which includes such a polypeptide as an active ingredient; or a pharmaceutical composition for promoting bone growth, having a therapeutically effective amount of such a polypeptide; or a method of increasing bone growth in a mammal by administering a therapeutically effective amount of such a polypeptide; having an amino acid sequence of a polypeptide defined in claim 56 or 57; or the use of such a polypeptide for the treatment of osteoporosis or to promote bone growth in a mammal; or the use of such a polypeptide in the preparation of a medicament for use in promoting bone growth or the treatment of osteoporosis; or a diagnostic kit for determining the presence of such a polypeptide, the kit including an antibody to a the polypeptide linked to a reporter system wherein the reporter system produces a detectable response when a predetermined amount of the polypeptide and the antibody are bound together; or an antibody which binds to such a polypeptide, synthesized using the polypeptide; or an isolated DNA fragment which encodes the expression of any such polypeptide, or which differs from the fragment due to the degeneracy of the genetic code; or a vector including a DNA sequence which encodes the expression of any such polypeptide; or a process for producing such a polypeptide, which process includes:

a) preparing a DNA fragment containing a nucleotide sequence which encodes the polypeptide;

b) incorporating the DNA fragment into an expression vector to obtain a recombinant DNA fragment which contains the DNA fragment and is capable of undergoing replication;

c) transforming a host cell with the recombinant DNA fragment to isolate a transformant which can express the polypeptide; and

d) culturing the transformant to allow the transformant to produce the polypeptide and recovering the polypeptide from resulting cultured mixture.

Finally, the invention includes an isolated DNA sequence encoding the amino acid sequence of any of the polypeptides of the invention, or an analogue thereof, wherein the amino acids in the sequence may be substituted, deleted or added, so long as bone stimulatory activity in mammals derived from the three dimensional conformation of the sequence is preserved in a polypeptide comprising the amino acid sequence; sequences which hybridize to the DNA and encode an amino acid sequence of a polypeptide which displays bone stimulatory activity in mammals; and DNA which differs from the sequence due to the degeneracy of the genetic code.

DESCRIPTION OF THE DRAWINGS

Figure 1 shows the bone apposition rate (μm per day) for rats injected with 25 nmol (N=5 in both cases) of chemically synthesized polypeptides having the sequences of NAP-2 (SEQ ID NO:1) and NAP-2V (SEQ ID NO:2), respectively, compared to that of a group of control (N=5) rats. The error bars are ± 1 S.D.

Figure 2 graphically illustrates the observed bone mineral apposition rate (μm per day) for rats injected with chemically synthesized polypeptides of **A**, NAP-2V-(1-26) (SEQ ID NO:11); **B**, NAP-2V-(13-26; gln^{25}-glu^{25}) (SEQ ID NO:12,), **C**, NAP-2V-(10-26) (SEQ ID NO:13), **D**, NAP-2V-(11-26) (SEQ ID NO:14), and **E**, NAP-2V-(12-26) (SEQ ID NO:15). The first bar in the graph represents the control. The number of rats used for the determinations were 4, 4, 3, 4, 4, and 4, respectively. The error bars are ± 1 S.D.

Figure 3 illustrates the amino acid sequences of polypeptides tested, corresponding amino acids aligned with each other, the active peptides being shown above the line and sequences which were not found to stimulate bone growth being below the line. Approximate molecular weights are shown below the sequence identification numbers.

MATERIALS, METHODS AND RESULTS

Polypeptides having the sequences of NAP-2 (SEQ ID NO:1) and NAP-2V (SEQ ID NO:2) were chemically synthesized directly according to standard methods and experiments were conducted to determine whether the chemically synthesized polypeptides displayed activity.

Experiments were conducted simultaneously on three groups of male Sprague-Dawley rats, there being five rats in each group. Each rate weighed between 250 and 350 g. Each rat of the first group was given, by subcutaneous injection into the left gluteus maximus region, 200 μl of a 1% aqueous acetic acid solution containing 25 nmol (about 191 μg) of NAP-2 (SEQ ID NO:1). Each rat of the second group was similarly given 200 μl of a 1% aqueous acetic acid solution containing 25 nmol (about 207 μg) of NAP-2V (SEQ ID NO:2). Each rat of the third group, the control group, was similarly given 200 μl of 1% acetic acid solution.

Immediately following administration of the test solution, 300 μl of an aqueous solution of tetracycline hydrochloride was administered intramuscularly into the right gluteus maximus, the concentration of tetracycline being sufficient to obtain a dosage of about 24mg/kg of rat body weight. A second dose of tetracycline hydrochloride solution was administered about 48 hours after the first dose. The rats were sacrificed about 24 hours after administration of the second dose of tetracycline.

Sections of the lower metaphysis of the right femur were used for bone measuring the bone mineral apposition rate. Processing of the bone material for

measurement has been described previously. See, for example, international patent application No. PCT/CA 94/00144 published under No. WO 94/20615 on September 15, 1994. The results obtained are summarized in Table One and Figure 1.

TABLE ONE: Comparison of the Group Arithmetic Means of Bone Apposition Rates (µm/day) Among Groups Administered with NAP-2, NAP-2V and control solutions shown in Figure 1

	Control	SEQ ID NO:1	SEQ ID NO:2
Mean	0.99 µm/d	1.23 µm/d	1.28 µm/d
S.D.	0.04	0.05	0.08
N	5	5	5
	t	d.f.	p
Control Group vs SEQ ID NO:1	7.91	8	<0.001
Control Group vs SEQ ID NO:2	7.03	8	<0.001
SEQ ID NO:1 vs SEQ ID NO:2	1.15	8	>0.20

Polypeptides having the sequence corresponding to either SEQ ID NO:1 or SEQ ID NO:2 have thus been found to stimulate bone growth.

In a second set of experiments, twenty-four male Sprague-Dawley rats (Charles River Laboratory) were divided into six groups of four. Each of the first group, the control group, was injected in the right thigh with 200 µl of 0.1% acetic acid solution. The rats of the other groups were each injected in the right thigh with about 200 µl of a 0.1% acetic acid solution containing about 25 nmol of a chemically synthesized polypeptide as follows:

Group	Polypeptide	SEQ ID NO
A	NAP-2V-(1-26)	11
B	NAP-2V-(13-26; gln^{25}-glu^{25})	12
C	NAP-2V-(10-26)	13
D	NAP-2V-(11-26)	14
E	NAP-2V-(12-26)	15

Immediately after injection of the polypeptide (control) solution, each rat was injected in the right gluteus maximus with 200 µl of a 1M tetracycline hydrochloride (Sigma Chemical) solution. This dosage of tetracycline is about 16 mg per kg of animal body weight.

About 48 hours later, each rat was injected in the left gluteus maximus with the same dosage of tetracycline. Twenty-four hours later, the rats were sacrificed by CO_2 narcosis and the right femur taken for bone mineral apposition rate determination. One rat died during the course of the experiments.

Immediately after dissection, a bone sample was fixed in 10% formaldehyde solution at pH 7.4. Later the same day, a 1:1 H_2O-acetone solution was exchanged for the formaldehyde solution. This was exchanged twice the following day with acetone. This was exchanged the following day by a 1:1 acetone-Spurr's medium solution, which was exchanged later the same day with Spurr's medium. The following day each sample was embedded in a fresh change of Spurr's medium and cured at 60°C for 24 hours, followed by curing at 80°C for 24 hours.

Each cured block was cut into 400 µm thick sections using a Leitz saw microtome equipped with a diamond charged blade. The relatively thick sections were ground down between two ground glass plates pre-roughened with carborundum powder to a final thickness of about 10 µm, water being used as the grinding lubricant. The thin sections were dried and mounted unstained in Permount (Fisher).

Measurements were made using a Leitz scanning light microscope photometer MPV-CD magnifying the sections 16X, as described in international patent application No. PCT/CA94/00144.

The results obtained are shown in Table Two and Figure 2.

Table TWO: Comparison of Group Arithmetic Means of Bone Apposition Rates (µm per day) shown in Figure 2.

	Control	Group A	Group B	Group C	Group D	Group E
Mean	1.08	1.47	1.70	1.18	1.07	1.04
S.D.	0.08	0.05	0.09	0.08	0.07	0.08
N	4	4	3	4	4	4

	t	d.f.	p
Control vs Group A	7.90	6	<0.001
Control vs Group B	9.69	5	<0.001
Group A vs Group B	4.87	5	

As graphically illustrated in Figure 2, NAP-2V-(1-26) (SEQ ID NO:11) and NAP-2V-(13-26; gln^{25}-glu^{25}) (SEQ ID NO:12) act to stimulate bone growth, the latter of these two polypeptides displaying greater activity.

NAP-2V-(10-26) (SEQ ID NO:13) appeared to cause a small increase in the observed bone apposition rate, although the significance of the observed increase was

questionable. NAP-2V-(11-26) (SEQ ID NO:14) and NAP-2V-(12-26) (SEQ ID NO:15) were found to have no effect on observed bone mineral apposition rate. The sequence of NAP-2V-(10-26) retains both the cys^{10} and cys^{12} residues. The sequences of NAP-2V-(11-26) and NAP-2V-(12-26) each retain the cys^{12} residue. The sequence of NAP-2V-(13-26; gln^{25}-glu^{25}) retains neither of the cys^{10} and cys^{12} residues. All of these NAP-2V subsequences lack the cys^{36} and cys^{52} residues present in the parent NAP-2V. It may be that the reduced activity of NAP-2V-(10-26), NAP-2V-(11-26) and NAP-2V-(12-26) is due to spontaneous intermolecular disulfide bonding that prevents a polypeptide-receptor interaction required for the bone stimulatory effect, but this is not known for certain.

The sequence of NAP-2V-(13-26; gln^{25}-glu^{25}) is different from the corresponding subsequence of NAP-2V at the 25 position, a glutamic acid residue being present in place of the glutamine residue. It would of course be expected that the subsequence having the glutamine residue as occurs in NAP-2V would also act to stimulate bone growth in mammals.

It has been postulated that NAP-2 contains two internal disulfide bonds, between Cys-5 and Cys-31, and Cys-7 and Cys-47, respectively (Baggiolini, M., Clemetson, K.J., Walz, A. International Patent Appication No. PCT/EP89/01389, published June 14, 1990 under WO90/06321.). By extension, sequences and subsequences disclosed herein that contain the corresponding cysteine residues would likely contain similar linkages therebetween.

It will of course be understood, without the intention of being limited thereby, that a variety of other substitutions of amino acids is possible while preserving the structure responsible for the bone stimulatory effect of the subsequences of NAP-2V disclosed herein. Conservative substitutions are described in the patent literature, as for example, in United States Patent No. 5,2264,558. It is thus expected, for example, that interchange among non-polar aliphatic neutral amino acids, glycine, alanine, proline, valine and isoleucine, would be possible. Likewise, substitutions among the polar aliphatic neutral amino acids, serine, threonine, methionine, asparagine and glutamine could possibly be made. Substitutions among the charged acidic amino acids, aspartic acid and glutamic acid, could probably be made, as could substitutions among the charged basic amino acids, lysine and arginine. Substitutions among the aromatic amino acids, including phenylalanine, histidine, tryptophan and tyrosine would also likely be possible. These sorts of substitutions and interchanges are well known to those skilled in the art. Other substitutions might well be possible. A peptide containing an amino acid sequence that can be aligned with that of SEQ ID NO:11 or SEQ ID NO:12 and having 50% or homology therewith may retain at least part of the bone stimulating effect thereof. Of course, it would also be expected that the greater percentage of homology,

say 60%, 70%, 80%, 90%, or more, could increase the degree of retained bone stimulating activity.

Insofar as deletion of one or more amino acids is concerned, it is likely that deletions of a small number of amino acids from each end of either sequence might be possible, bearing in mind the observation that the deletions to obtain SEQ ID NOs: 14 and 15 yield polypeptides which do not appear to enhance bone growth. Further, symmetrical, or nearly symmetrical deletions would likely be the most possible to be made while retaining the three-dimensional configuration. Internal deletions, although likely to be possible to some limited extent, should be few.

Additions of amino acids could very likely be made at the ends of the sequence, and as with deletions, symmetrical or nearly symmetrical additions to the carboxy and amino terminals are likely to be possible. Internal additions, although likely to be possible to some limited extent, should be few.

Of the above-listed modifications to the sequence, terminal additions are most likely to be most useful, as such a modification can serve a variety of functions: an identifying group as for use in a radioimmunoassay; or a linking group, as examples.

A polypeptide of the present invention can be improved with respect to possible degradation, as might occur in the body in the presence of protease, for instance, by protection of the C-terminus, the N-terminus, or both the C-terminus and N-terminus of the polypeptide.

As used herein, "protected" terminal amino group refers to a terminal amino group (N-terminus) coupled with any of various amino-terminal protecting groups that can be employed in peptide synthesis. Examples of suitable groups include acyl protecting groups, for example, formyl, acetyl, benzoyl, trifluoroacetyl, succinyl, and methoxysuccinyl; aromatic urethane protecting groups, for example benzyloxycarbonyl; and aliphatic urethane protecting groups, for example *t*-butoxycarbonyl or adamantyloxycarbonyl (Gross and Mienhofer, eds., *The Peptides*, vol 3, pp. 3 to 88 (Academic Press, New York, 1981)).

As used herein, "protected" terminal carboxyl group refers to a terminal carboxyl group (C-terminus) coupled with any of various carboxy-terminal protecting groups. As will be readily apparent to a person skilled in the art, suitable groups include *t*-butyl, benzyl or other acceptable groups linked to the terminal carboxyl group through an ester or ether bond.

Compounds within the scope of this invention can be synthesized chemically be means well known in the art such, for example, solid phase peptide synthesis. The synthesis is commenced from the carboxy-terminal end of the peptide using an α-amino protected amino acid. *t*-Butyloxycarbonyl (Boc) protective groups, or other suitable protective groups, can be used (Stewart *et al.*, "Solid-Phase Peptide Synthesis," W. H. Freeman Co.,

San Francisco (1969); Merrifield, *J. Am. Chem. Soc.* 85:2149-2154 (1963); Vale *et al.*, *Science* 213, 1394-1397 (1981), and Marke *et al. J. Am Chem. Sci.* 103, 3178 (1981)). Synthetic methods are also described in "Principles of Peptide Synthesis" M. Bodansky Ed. (Spring-Verlag 1984). These and other methods of peptide synthesis are also exemplified by U.S. Patent Nos. 3,862,925, 3,842,067, 3,972,859, 4,105,602, 4,683,291, 4,244,946 and 4,305,872.

Compounds may also be synthesized using manual or automatic techniques, for example, an Applied BioSystems 430A Peptide Synthesizer (Foster City, California) or a Biosearch SAM 11 automatic peptide synthesizer (Biosearch, Inc., San Rafael, California).

Compounds of the present invention and compositions containing them find use in numerous therapeutic and prophylactic applications in the prevention and treatment of bone reduction related to a disease. Compounds can thus be used as treatments to promote bone growth, in the treatment of osteoporosis, for example, by any suitable route. The preferred routes are suitable for delivery of polypeptide-type compounds to the bloodstream of a subject, bearing in mind proper storage and handling conditions required for polypeptides such as those described herein.

Thus the present invention also provides compositions containing an effective amount of compounds of the present invention, including the nontoxic addition salts, amides and esters thereof, which may, alone, serve to provide the treatment benefits described above. Such compositions can also be provided together with physiologically tolerable liquid, gel or solid diluents, adjuvants and excipients.

In the above examples involving subsequences of NAP-2V, about 75 nmol of polypeptide per kg of bodyweight of animal was used per administration. In practice, particularly as human subjects are concerned, the daily dosage may well be between 0.01 and 300 mg or more per kg of bodyweight. More preferably, the dosage would be in the neighbourhood of from about 0.1 to about 30 mg per kg of bodyweight. It may be that the preferred frequency of administration would be greater or less than once per day, depending upon the route of administration, convenience, and the variation of effectiveness of treatment with frequency of and amount used per administration. The dosage administered also depends on the subject and to which effect such administration is to give. The dosage of any one or more of the compounds will depend on many factors including the specific compound or combination of compounds being utilized, the mode of administration, and the mammal being treated. Dosages of a particular compound or combination of compounds can be determined using conventional considerations; for example, by customary comparison of the differential activities of the subject compounds and that of a known agent, that is, by means of an appropriate pharmacological protocol in which, for example, bone density of subjects is measured over time.

Pharmaceutical preparations include any of the compounds prepared as an injectable solution, including an injectable solution prepared just prior to use, for promoting bone growth and/or treatment of osteoporosis. An injectable can be either a liquid solution or suspension; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified. The active polypeptide is often mixed with diluents and excipients which are physiologically tolerable and compatible with the polypeptide. Suitable diluents and excipients are, for example, water, saline, dextrose, glycerol, or the like, and combinations thereof. In addition, if desired, the compositions can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, stabilizing or pH buffering agents, and the like.

Pharmaceutical preparations include the employment of the compounds in admixture with conventional excipients, that is, pharmaceutically acceptable organic or inorganic carrier substances which do not deleteriously react with the compounds, and which possibly enhance the storage and handling stability of the compounds. The preparative procedure may include the sterilization of the pharmaceutical preparations. The compounds may be mixed with auxiliary agents such as lubricants, preservatives, stabilizers, salts for influencing osmotic pressure, etc., which do not react deleteriously with the compounds.

The compositions are conventionally administered parenterally, by injection, for example either subcutaneously or intravenously. Additional formulations which are suitable for other modes of administration include suppositories, intranasal aerosols, and, in some cases, oral formulations. For suppositories, traditional binders and excipients may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills capsules, sustained release formulations, or powders, and contain 10% - 95% of active ingredient, preferably 25% - 70%. These oral formulations include formulations designed to protect the peptide until it can be absorbed.

The peptide compounds may be formulated into the compositions as neutral or salt forms. Pharmaceutically acceptable non-toxic salts include the acid addition salts (formed with the free amino groups) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The compounds of the invention can be homopolymerized to themselves (i.e., $(peptide)_n$) or, heteropolymerized to one another. The compounds can also be conjugated to biocompatible polymeric compounds, such as BIOPOL™ (WR Grace & Co.-Conn.).

If prepared using recombinant techniques, a DNA sequence encoding a desired polypeptide of the present invention is synthesized using standard automated techniques, or the coding sequences or portions thereof are retrieved from cDNA or genomic libraries. This DNA is ligated into suitable expression vectors and these vectors are transformed into appropriate hosts. A variety of expression vector/host cell systems can be used, including both procaryotic and eukaryotic culture systems.

Procaryotes most frequently are represented by various strains of E. coli. However, other microbial strains may also be used, such as bacilli, for example *bacillus subtilis*, various species of *Pseudomonas*, or other bacterial strains. In such procaryotic systems, plasmid vectors which contain replication origins, and control sequences derived from a species compatible with the host are used. For example, E. coli is typically transformed using derivatives of pBR322, a plasmid derived from an E. coli species (Bolivar et al., *(1977) Gene* **2**:95. Commonly used procaryotic control sequences, which are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences, include such commonly used promoters as the beta-lactamase (penicillinase), lactose (1nc) promoter systems (Chang et al., *(1977) Nature* **198**:1056), the tryptophan (trp) promoters system (Goeddel et al., (1990) *Nucleic Acids Res* 8:4057), and the lambda-derived P_L promoter and N-gene ribosome binding site (Shimatake et al., (1981) *Nature* **292**:128). However, any available promoter system compatible with procaryotes can be used.

The expression systems useful in the eukaryotic systems of the invention comprise promoters derived from appropriate eukaryotic genes. A class of promoters useful in yeast, for example, include promoters for synthesis of glycolytic enzymes, including alcohol dehydrogenase promoters, glyceraldehyde-3-phosphate dehydrogenase promoter (Holland & Holland, (1980) *J Biol Chem* **25**:2596), alpha-factor promoter (Bitter et al., (1984) *Proc Natl Acad Sci* **81**:5330), the gal promoter (Johnston & David, (1984) *Mol Cell Biol* **4**:1440) those for 3-phosphoglycerate kinase (Hitzeman et al., (1980) *J. Biol Chem* **256**:1385) or the Leu2 gene obtained from YEp13 (Broach, J., et al., (1978) *Gene* **8**:121).

Suitable mammalian promoters include the early and late promoters from SV40 (Fiers et al., (1978) *Nature* **273**:113) or other viral promoters such as those derived from polyoma, adenovirus II, bovine papilloma virus or avian sarcoma viruses. Suitable viral and mammalian enhancers are cited above. In the event plant cells are used as an expression system, the nopaline synthesis promoter is appropriate (Depicker, A., et al., (1982) *J Mol Appl Gen* **1**:56).

The expression systems are included on replication vectors or are caused to integrate into the chromosome of a recombinant host. For systems wherein the vectors include a replication system, these may be low or high copy number, usually having copy numbers of fewer than about 1000, although in certain situations, runaway vectors may be employed. Whether provided on a vector intended for integration or in a replication system, the sequence encoding a polypeptide of the invention may be ligated in tandem with an amplifying gene such as dihydrofolate reductase, metallothioneins, thymidine kinase, or the like. In procaryotic systems, both the amplifying gene and the target gene can be under the regulation of the same transcriptional and translational regulatory regions.

Usually, the vector will include a marker which allows for selection of host cells containing the expression system; the nature of these markers depends on the host and is understood in the art. In addition to required regulators such as promoters, additional sequences such as enhancers can also be employed to enhance the level of transcription. If the polypeptide is to be secreted, an upstream sequence encoding signal peptides such as those described in U.S. Pat. Nos. 4,336,336; 4,338,397; and 4,546,082 may be employed. The signal sequence is enzymatically cleaved as the polypeptide product is secreted.

Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described by Cohen, S.N., (1972) *Proc Natl Acad Sci USA* **69**:2110; or the RbCl method described in Maniatis et al., *Molecular Cloning: A Laboratory Manual* (1982) Cold Spring Harbor Press, p. 254 is used for procaryotes or other cells which contain substantial cell wall barriers. Infection with *Agrobacterium tumefaciens* (Shaw, C.H., (1938) et al., *Gene* **23**:315) is used for certain plant cells. For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, (1978) *Virology* **52**:2:546 is preferred. Transformations into yeast are carried out, for example, according to the method of Van Solingen, P., et al., (1977) *J Bacter* **130**:946; and Hsiao, C.L., et al., (1979) *Proc Natl Acad Sci* USA **76**:3829.

In general, after construction of a suitable expression system, the system is transfected into the appropriate host and successful transformants are selected by markers contained on the expression vectors. Successfully transformed colonies are then cultured in order to produce the desired polypeptide. It is sometimes preferred that a promoter which can be controlled by regulating conditions in the environment be used so that the cells can be grown under conditions where the gene encoding the desired polypeptide of the invention is not expressed, and then production of the polypeptide induced by appropriate manipulation of conditions. For example, if the trp promoter is used in E. coli, the cells are grown in the presence of tryptophan and expression is then induced by diminution of tryptophan concentration or by addition of a tryptophan analogue such as indolylacetic acid. If the gene is

under control of the PL promoter, the cells are grown at relatively low temperature, such as at about 35°C., to a suitable cell density, and the temperature is then elevated to activate this promoter. If produced in bacterial hosts as a mature intracellular polypeptide, the N-terminal methionine may or may not be cleaved. In mammalian systems, for example, the use of the metallothionein promoter permits induction by addition of heavy metals or glucocorticoids. This protocol is preferred to prevent premature accumulation of the polypeptide which might be harmful to the growth of the cell.

The polypeptide can be produced intracellularly, or in secreted form by construction of vectors in which the peptide is preceded by a signal peptide workable in the appropriate host.

The polypeptide is recovered from the medium or from the cells using suitable techniques generally known in the art, and purified by, for example, ion exchange chromatography, ammonium sulfate precipitation, gel permeation chromatography, and so forth.

The polypeptide made available by the invention disclosed herein can be used to obtain antisera thereto (Stites, D.P. and A.I. Terr. 1991. In Basic & Clinical Immunology, 7th Ed. Appleton and Lange, Norwalk, Connecticut and San Matea California). Methodology and products can be developed using an antibody to a polypeptide for use in detecting the polypeptide with which the antibody binds. This apparently having been accomplished at least for the polypeptide having the sequence of CTAP-III (SEQ ID NO:3) (Baggiolini, M., Clemetson, K.J., Walz, A. International Patent Appication No. PCT/EP89/01389, published June 14, 1990 under WO90/06321). Methodology and products can be developed using an antibody to a polypeptide for use in detecting the polypeptide with which the antibody binds.

For example, an antibody can be linked to or conjugated with a reporter system which is set up to indicate positively binding of the polypeptide to the antibody. Well known reporter systems include radioimmuno assays (RIAs) or immunoradiometric assays (IRMAs). Alternatively, an enzyme-linked immunosorbent assay (ELISA) would have in common with RIAs and IRMAs a relatively high degree of sensitivity, but would generally not rely upon the use of radioisotopes. A visually detectable substance may be produced or at least one detectable in a spectrophotometer. An assay relying upon fluroescence of a substance bound by the enzyme being assayed could be used. It will be appreciated that there are a number of reporter systems which may be used, according to the present invention, to detect the presence of a particular polypeptide. With standardized sample collection and treatment, polypeptide presence above a threshold amount in blood serum could well be determined.

Such an antibody-linked reporter system could be used in a method for determining whether blood serum of a subject contains a deficient amount of the polypeptide. Given a normal threshold concentration of such a polypeptide in blood serum of a given type of subject, test kits could thus be developed.

A further advantage may be obtained through chimeric forms of the protein, as known in the art. A DNA sequence encoding the entire protein, or a portion of the protein, could thus be linked with a sequence coding for the C-terminal portion of *E. coli* ß-galactosidase to produce a fusion protein, for example. An expression system for human respiratory syncytial virus glycoproteins F and G is described in United States Patent No. 5,288,630 issued February 22, 1994 and references cited therein, for example.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
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(F) POSTAL CODE (ZIP) : L6M 2B7

(ii) TITLE OF INVENTION: BONE STIMULATING FACTOR

(iii) NUMBER OF SEQUENCES: 16

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette, 3 1/2 inch, 1.4 Mb storage
(B) COMPUTER: COMPAQ, IBM PC compatible
(C) OPERATING SYSTEM: MS-DOS 5.1
(D) SOFTWARE: WORD PERFECT

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NO:
(B) FILING DATE: 26-SEPTEMBER-1996
(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 60/004,314
(B) FILING DATE: 26-SEPTEMBER-1995

(2) INFORMATION FOR SEQ ID NO:1
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1

Ala Glu Leu Arg Cys Met Cys Ile Lys Thr Thr Ser Gly Ile His Pro
1 5 10 15

Lys Asn Ile Gln Ser Leu Glu Val Ile Gly Lys Gly Thr His Cys Asn
20 25 30

Gln Val Glu Val Ile Ala Thr Leu Lys Asp Gly Arg Lys Ile Cys Leu
35 40 45

Asp Pro Asp Ala Pro Arg Ile Lys Lys Ile Val Gln Lys Lys Leu Ala
50 55 60

Gly Asp Glu Ser Ala Asp
65 70

(2) INFORMATION FOR SEQ ID NO:2
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 75 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2

Asp Ser Asp Leu Tyr Ala Glu Leu Arg Cys Met Cys Ile Lys Thr Thr
1 5 10 15

Ser Gly Ile His Pro Lys Asn Ile Gln Ser Leu Glu Val Ile Gly Lys
20 25 30

Gly Thr His Cys Asn Gln Val Glu Val Ile Ala Thr Leu Lys Asp Gly
35 40 45

Arg Lys Ile Cys Leu Asp Pro Asp Ala Pro Arg Ile Lys Lys Ile Val
50 55 60

Gln Lys Lys Leu Ala Gly Asp Glu Ser Ala Asp
65 70 75

(2) INFORMATION FOR SEQ ID NO:3

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 74 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3

```
Ser Asp Leu Tyr Ala Glu Leu Arg Cys Met Cys Ile Lys Thr Thr Ser
 1               5                  10                  15

Gly Ile His Pro Lys Asn Ile Gln Ser Leu Glu Val Ile Gly Lys Gly
            20                  25                  30

Thr His Cys Asn Gln Val Glu Val Ile Ala Thr Leu Lys Asp Gly Arg
        35                  40                  45

Lys Ile Cys Leu Asp Pro Asp Ala Pro Arg Ile Lys Lys Ile Val Gln
    50                  55                  60

Lys Lys Leu Ala Gly Asp Glu Ser Ala Asp
65                  70
```

(2) INFORMATION FOR SEQ ID NO:4

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 73 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4

```
Asp Leu Tyr Ala Glu Leu Arg Cys Met Cys Ile Lys Thr Thr Ser Gly
 1               5                  10                  15

Ile His Pro Lys Asn Ile Gln Ser Leu Glu Val Ile Gly Lys Gly Thr
            20                  25                  30

His Cys Asn Gln Val Glu Val Ile Ala Thr Leu Lys Asp Gly Arg Lys
        35                  40                  45

Ile Cys Leu Asp Pro Asp Ala Pro Arg Ile Lys Lys Ile Val Gln Lys
    50                  55                  60

Lys Leu Ala Gly Asp Glu Ser Ala Asp
65                  70
```

(2) INFORMATION FOR SEQ ID NO:5
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5

Gly Lys Glu Glu Ser Leu Asp Ser Asp Leu Tyr Ala Glu Leu Arg Cys
1 5 10 15

Met Cys Ile Lys Thr Thr Ser Gly Ile His Pro Lys Asn Ile Gln Ser
20 25 30

Leu Glu Val Ile Gly Lys Gly Thr His Cys Asn Gln Val Glu Val Ile
35 40 45

Ala Thr Leu Lys Asp Gly Arg Lys Ile Cys Leu Asp Pro Asp Ala Pro
50 55 60

Arg Ile Lys Lys Ile Val Gln Lys Lys Leu Ala Gly Asp Glu Ser Ala
65 70 75 80

Asp

(2) INFORMATION FOR SEQ ID NO:6
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6

Asn Leu Ala Lys Gly Lys Glu Glu Ser Leu Asp Ser Asp Leu Tyr Ala
1 5 10 15

Glu Leu Arg Cys Met Cys Ile Lys Thr Thr Ser Gly Ile His Pro Lys
20 25 30

Asn Ile Gln Ser Leu Glu Val Ile Gly Lys Gly Thr His Cys Asn Gln
35 40 45

Val Glu Val Ile Ala Thr Leu Lys Asp Gly Arg Lys Ile Cys Leu Asp
50 55 60

Pro Asp Ala Pro Arg Ile Lys Lys Ile Val Gln Lys Lys Leu Ala Gly
65 70 75 80

Asp Glu Ser Ala Asp
85

(2) INFORMATION FOR SEQ ID NO:7
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 94 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7

Ser Ser Thr Lys Gly Gln Thr Lys Art Asn Leu Ala Lys Gly Lys Glu
1 5 10 15

Glu Ser Leu Asp Ser Asp Leu Tyr Ala Glu Leu Arg Cys Met Cys Ile
20 25 30

Lys Thr Thr Ser Gly Ile His Pro Lys Asn Ile Gln Ser Leu Glu Val
35 40 45

Ile Gly Lys Gly Thr His Cys Asn Gln Val Glu Val Ile Ala Thr Leu
50 55 60

Lys Asp Gly Arg Lys Ile Cys Leu Asp Pro Asp Ala Pro Arg Ile Lys
65 70 75 80

Lys Ile Val Gln Lys Lys Leu Ala Gly Asp Glu Ser Ala Asp
85 90

(2) INFORMATION FOR SEQ ID NO:8
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 79 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8

Glu Gly Ala Val Leu Pro Arg Ser Ala Lys Glu Leu Arg Cys Gln Cys
1 5 10 15

Ile Lys Thr Tyr Ser Lys Pro Phe His Pro Lys Phe Ile Lys Glu Leu
20 25 30

Arg Val Ile Glu Ser Gly Pro His Cys Ala Asn Thr Glu Ile Ile Val
35 40 45

Lys Leu Ser Asp Gly Arg Glu Leu Cys Leu Asp Pro Lys Glu Asn Trp
50 55 60

Val Gln Arg Val Val Glu Lys Phe Leu Lys Arg Ala Glu Asn Ser
65 70 75

(2) INFORMATION FOR SEQ ID NO:9
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 103 base pairs
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9

Met Thr Ser Lys Leu Ala Val Ala Phe Leu Ala Val Phe Leu Leu Ser
1 5 10 15

Ala Ala Leu Cys Glu Ala Asp Val Leu Ala Arg Val Ser Ala Glu Leu
20 25 30

Arg Cys Gln Cys Ile Asn Thr His Ser Thr Pro Phe His Pro Lys Phe
35 40 45

Ile Lys Gle Leu Arg Val Ile Gle Ser Gly Phe His Cys Glu Asn Ser
50 55 60

Glu Ile Ile Val Lys Leu Val Asn Gly Lys Glu Val Cys Leu Asp Pro
65 70 75 80

Lys Glu Lys Trp Val Gln Lys Val Val Gln Ile Phe Leu Lys Arg Thr
85 90 95

Glu Lys Gln Gln Gln Gln Gln
100

(2) INFORMATION FOR SEQ ID NO:10
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 70 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10

Glu Ala Glu Glu Asp Gly Asp Leu Gln Cys Leu Cys Val Lys Thr Thr
1 5 10 15

Ser Gln Val Arg Pro Arg His Ile Thr Ser Leu Glu Val Ile Lys Ala
20 25 30

Gly Pro His Cys Pro Thr Ala Gln Leu Ile Ala Thr Leu Lys Asn Gly
35 40 45

Arg Lys Ile Cys Leu Asp Leu Glu Ala Pro Leu Tyr Lys Lys Ile Ile
50 55 60

Lys Lys Leu Leu Glu Ser
65 70

(2) INFORMATION FOR SEQ ID NO:11
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11

Asp Ser Asp Leu Tyr Ala Glu Leu Arg Cys Met Cys Ile Lys Thr Thr
1 5 10 15

Ser Gly Ile His Pro Lys Asn Ile Gln Ser
20 25

(2) INFORMATION FOR SEQ ID NO:12
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12

Ile Lys Thr Thr Ser Gly Ile His Pro Lys Asn Ile Glu Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:13
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13

Cys Met Cys Ile Lys Thr Thr Ser Gly Ile His Pro Lys Asn Ile Gln
1 5 10 15

Ser

(2) INFORMATION FOR SEQ ID NO:14
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14

Met Cys Ile Lys Thr Thr Ser Gly Ile His Pro Lys Asn Ile Gln Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15

```
Cys Ile Lys Thr Thr Ser Gly Ile His Pro Lys Asn Ile Gln Ser
 1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:16

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 228 base pairs

(B) TYPE: nucleic acid

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16

```
GAC AGT GAC TTG TAT GCT GAA CTC CGC TGC ATG TGT ATA AAG ACA ACC      48
Asp Ser Asp Leu Tyr Ala Glu Leu Arg Cys Met Cys Ile Lys Thr Thr
 1               5                  10                  15

TCT GGA ATT CAT CCC AAA AAC ATC CAA AGT TTG GAA GTG ATC GGG AAA      96
Ser Gly Ile His Pro Lys Asn Ile Gln Ser Leu Glu Val Ile Gly Lys
            20                  25                  30

GGA ACC CAT TGC AAC CAA GTC GAA GTC ATA GCC ACA CTG AAG GAT GGG     146
Gly Thr His Cys Asn Gln Val Glu Val Ile Ala Thr Leu Lys Asp Gly
        35                  40                  45

AGG AAA ATC TGC CTG GAC CCA GAT GCT CCC AGA ATC AAG AAA ATT GTA     192
Arg Lys Ile Cys Leu Asp Pro Asp Ala Pro Arg Ile Lys Lys Ile Val
    50                  55                  60

CAG AAA AAA TTG GCA GGT GAT GAA TCT GCT GAT TAA                     228
Gln Lys Lys Leu Ala Gly Asp Glu Ser Ala Asp TER
65                  70                  75
```

CLAIMS

1. A polypeptide which promotes bone growth in mammals, comprising an amino acid sequence corresponding to SEQ ID NO:2 with (a) from 6 to about 12 amino acids deleted from the N-terminus of SEQ ID NO:2, (b) 7 to about 49 amino acids deleted from the C-terminus of SEQ ID NO:2, or both (a) and (b); wherein the sequence includes no cysteine residues or at least two cysteine residues; or a functionally equivalent homologue.

2. A polypeptide which promotes bone growth in mammals, comprising an amino acid sequence corresponding to SEQ ID NO:11 up to 69 amino acids in length, or corresponding to SEQ ID NO:11 with from 6 to about 12 amino acids deleted from the N-terminus of SEQ ID NO:11, or corresponding to SEQ ID NO:11 with from 6 to about 9 amino acids deleted from the N-terminus of SEQ ID NO:11; wherein the sequence includes no cysteine residues or at least two cysteine residues; or a functionally equivalent homologue.

3. A polypeptide which promotes bone growth in mammals, comprising an amino acid sequence corresponding to SEQ ID NO:12 up to 69 amino acids in length; or a functionally equivalent homologue.

4. A polypeptide comprising an amino acid sequence consisting essentially of the amino acid sequence corresponding to SEQ ID NO:11; or a conservatively substituted variant thereof.

5. A polypeptide comprising an amino acid sequence consisting essentially of the amino acid sequence corresponding to SEQ ID NO:12; or a conservatively substituted variant thereof.

6. A polypeptide comprising an amino acid sequence consisting essentially of the amino acid sequence corresponding to SEQ ID NO:13; or a conservatively substituted variant thereof.

7. The polypeptide of claim 1 wherein the at least two cysteine residues are located at positions corresponding to the tenth and twelfth positions of SEQ ID NO:2.

8. The polypeptide of claim 1 wherein any substitution of an amino acid is a conservative substitution.

9. The polypeptide of any of claims 1 to 8 wherein one or the other or both of the N-terminal amino acid and the C-terminal amino acid includes a protecting group.

10. A polypeptide of any of claims 1 to 8 wherein the polypeptide is synthetic and the amino acid sequence has a molecular weight in the range of from about 1000 to 4000.

11. A polypeptide of any of claims 1 to 8 wherein the amino acid sequence has a molecular weight in the range of from about 1500 to about 3000.

12. A polypeptide of any of claims 1 to 8 wherein the amino acid sequence has a molecular weight in the range of from about 1500 to about 1800.

13. A first polypeptide comprising a sequence of amino acids up to 69 amino acids in length and sufficiently duplicative of a second polypeptide comprising an amino acid sequence corresponding to SEQ ID NO:2 with (a) from 6 to about 12 amino acids deleted from the N-

terminus of SEQ ID NO:2, (b) 7 to about 49 amino acids deleted from the C-terminus of SEQ ID NO:2, or both (a) and (b); wherein the sequence includes no cysteine residues or at least two cysteine residues; or a functionally equivalent homologue, such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide.

14. A first polypeptide comprising a sequence of amino acids up to 69 amino acids in length and sufficiently duplicative of a second polypeptide comprising an amino acid sequence corresponding to SEQ ID NO:11 up to 69 amino acids in length, or corresponding to SEQ ID NO:11 with from 6 to about 12 amino acids deleted from the N-terminus of SEQ ID NO:11, or corresponding to SEQ ID NO:11 with from 6 to about 9 amino acids deleted from the N-terminus of SEQ ID NO:11; wherein the sequence includes no cysteine residues or at least two cysteine residues; or a functionally equivalent homologue, such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide.

15. A first polypeptide comprising a sequence of amino acids up to 69 amino acids in length and sufficiently duplicative of a second polypeptide comprising an amino acid sequence corresponding to SEQ ID NO:12 up to 69 amino acids in length; or a functionally equivalent homologue, such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide.

16. A first polypeptide comprising a sequence of amino acids up to 69 amino acids in length and sufficiently duplicative of a second polypeptide comprising an amino acid sequence corresponding to to SEQ ID NO:11; or a conservatively substituted variant thereof, such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide.

17. A first polypeptide comprising a sequence of amino acids up to 69 amino acids in length and sufficiently duplicative of a second polypeptide comprising an amino acid sequence corresponding to SEQ ID NO:12; or a conservatively substituted variant thereof, such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide.

18. A first polypeptide comprising a sequence of amino acids up to 69 amino acids in length and sufficiently duplicative of a second polypeptide comprising an amino acid sequence corresponding to SEQ ID NO:13; or a conservatively substituted variant thereof, such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide.

19. A first polypeptide of any of claims 13 to 18, up to 60 amino acids in length.

20. A first polypeptide of any of claims 13 to 18, up to 50 amino acids in length.

21. A first polypeptide of any of claims 13 to 18, up to 40 amino acids in length.

22. A first polypeptide of any of claims 13 to 18, up to 30 amino acids in length.

23. A first polypeptide of any of claims 13 to 18, up to 20 amino acids in length wherein the amino acid sequence includes no cysteine residues or at least two cysteine residues.

24. A first polypeptide of any of claims 13 to 18, up to 14 amino acids in length.

25. A chimeric bone stimulating factor comprising an amino acid sequence of any of claims 1 to 8 or claims 13 to 18.

26. An agent for use in prevention and treatment of a bone reduction related disease which comprises a polypeptide of any of claims 1 to 8 or claims 13 to 18 as an active ingredient.

27. A pharmaceutical composition for promoting bone growth, comprising a therapeutically effective amount of a polypeptide of any of claims 1 to 8 or claims 13 to 18.

28. A method of increasing bone growth in a mammal by administering a therapeutically effective amount of a polypeptide having an amino acid sequence of a polypeptide defined in any of claims 1 to 8 or claims 13 to 18.

29. The use of a polypeptide of any of claims 1 to 8 or claims 13 to 18 for the treatment of osteoporosis.

30. The use of a polypeptide of any of claims 1 to 8 or claims 13 to 18 to promote bone growth in a mammal.

31. The use of a polypeptide having a sequence according to any of claims 1 to 8 or claims 13 to 18 in the preparation of a medicament for use in promoting bone growth or the treatment of osteoporosis.

32. A diagnostic kit for determining the presence of a polypeptide of any of claims 1 to 8 or claims 13 to 18 comprising an antibody to a said polypeptide linked to a reporter system wherein the reporter system produces a detectable response when a predetermined amount of the polypeptide and the antibody are bound together.

33. An antibody synthesized using a polypeptide consisting of an amino acid sequence identified as SEQ ID NO:11; SEQ ID NO:12; or SEQ ID NO:13 or a conservatively substituted variant thereof.

34. An antibody which binds to a polypeptide defined in any of claims 1 to 8 or claims 13 to 18, synthesized using the polypeptide.

35. An isolated DNA fragment which encodes the expression of any of the polypeptides of claims 1 to 8 or claims 13 to 18, and DNA which differs from the fragment due to the degeneracy of the genetic code.

36. A vector comprising a DNA sequence which encodes the expression of any of the polypeptides of claims 1 to 7 or 9.

37. A process for producing a polypeptide of any of claims 1 to 8 or claims 13 to 18, which comprises:

a) preparing a DNA fragment containing a nucleotide sequence which encodes said polypeptide;

b) incorporating said DNA fragment into an expression vector to obtain a recombinant DNA fragment which contains said DNA fragment and is capable of undergoing replication;

c) transforming a host cell with said recombinant DNA fragment to isolate a transformant which can express said polypeptide; and

d) culturing said transformant to allow the transformant to produce said polypeptide and recovering said polypeptide from resulting cultured mixture.

38. A synthetic polypeptide up to 65 amino acids in length, having *in vivo* bone stimulatory activity in mammals, having an amino acid sequence which is at least about 19% conserved in relation to the amino acid sequence identified as SEQ ID NO:2; a conservatively substituted variant thereof; or a functionally equivalent homologue.

39. A synthetic polypeptide up to 65 amino acids in length, having *in vivo* bone stimulatory activity in mammals, having an amino acid sequence which is at least about 22% conserved in relation to the amino acid sequence identified as SEQ ID NO:2; a conservatively substituted variant thereof; or a functionally equivalent homologue.

40. A synthetic polypeptide up to 65 amino acids in length, having *in vivo* bone stimulatory activity in mammals, having an amino acid sequence which is at least about 25% conserved in relation to the amino acid sequence identified as SEQ ID NO:2; a conservatively substituted variant thereof; or a functionally equivalent homologue.

41. A synthetic polypeptide up to 65 amino acids in length, having *in vivo* bone stimulatory activity in mammals, having an amino acid sequence which is at least about 28% conserved in relation to the amino acid sequence identified as SEQ ID NO:2; a conservatively substituted variant thereof; or a functionally equivalent homologue.

42. A synthetic polypeptide up to 65 amino acids in length, having *in vivo* bone stimulatory activity in mammals, having an amino acid sequence which is at least about 31% conserved in relation to the amino acid sequence identified as SEQ ID NO:2; a conservatively substituted variant thereof; or a functionally equivalent homologue.

43. A synthetic polypeptide up to 65 amino acids in length, having *in vivo* bone stimulatory activity in mammals, having an amino acid sequence which is at least about 35% conserved in relation to the amino acid sequence identified as SEQ ID NO:2; a conservatively substituted variant thereof; or a functionally equivalent homologue.

44. A polypeptide of claim 38, 39, 40, 41, 42 or 43 having at least 49 amino acids deleted from said sequence.

45. A polypeptide of claim 38 or 39, having at least 58 amino acids deleted from said sequence.

46. A polypeptide of claim 38 having at least 61 amino acids deleted from said sequence.

47. A polypeptide of claim 38, 39, 40, 41, 42 or 43 wherein the sequence includes no cysteine residues or at least two cysteine residues.

48. A polypeptide of claim 29, 30, 31 or 32 wherein the polypeptide has a molecular weight in the range of from about 1000 to 4000.

49. A first polypeptide comprising a sequence of amino acids up to 69 amino acids in length and sufficiently duplicative of a second polypeptide comprising an amino acid sequence of claim 38, 39, 40, 41, 42 or 43 such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide.

50. A first polypeptide of claim 49, up to 60 amino acids in length.

51. A first polypeptide of claim 49, up to 50 amino acids in length.

52. A first polypeptide of claim 49, up to 40 amino acids in length.

53. A first polypeptide of claim 49, up to 30 amino acids in length.

54. A first polypeptide of claim 49, up to 20 amino acids in length wherein the amino acid sequence includes no cysteine residues or at least two cysteine residues.

55. A first polypeptide of claim 49, up to 14 amino acids in length.

56. A chimeric bone stimulating factor comprising an amino acid sequence of claim 38, 39, 40, 41, 42 or 43.

57. An agent for use in prevention and treatment of a bone reduction related disease which comprises a polypeptide of claim 38, 39, 40, 41, 42 or 43.

58. A pharmaceutical composition for promoting bone growth, comprising a therapeutically effective amount of a polypeptide of claim 38, 39, 40, 41, 42 or 43.

59. A method of increasing bone growth in a mammal by administering a therapeutically effective amount of a polypeptide having an amino acid sequence of a polypeptide defined in claim 38, 39, 40, 41, 42 or 43.

60. The use of a polypeptide of claim 38, 39, 40, 41, 42 or 43 for the treatment of osteoporosis.

61. The use of a polypeptide of claim 38, 39, 40, 41, 42 or 43 to promote bone growth in a mammal.

62. The use of a polypeptide having a sequence according to claim 38, 39, 40, 41, 42 or 43 in the preparation of a medicament for use in promoting bone growth or the treatment of osteoporosis.

63. A diagnostic kit for determining the presence of a polypeptide of claim 38, 39, 40, 41, 42 or 43 comprising an antibody to a said polypeptide linked to a reporter system wherein the reporter system produces a detectable response when a predetermined amount of the polypeptide and the antibody are bound together.

64. An antibody which binds to a polypeptide defined in claim 38, 39, 40, 41, 42 or 43, synthesized using the polypeptide.

65. An isolated DNA fragment which encodes the expression of any of the polypeptides of claim 38, 39, 40, 41, 42 or 43, and DNA which differs from the fragment due to the degeneracy of the genetic code.

66. A vector comprising a DNA sequence which encodes the expression of any of the polypeptides of claim 38, 39, 40, 41, 42 or 43.

67. A process for producing a polypeptide of any of claim 38, 39, 40, 41, 42 or 43, which comprises:

a) preparing a DNA fragment containing a nucleotide sequence which encodes said polypeptide;

b) incorporating said DNA fragment into an expression vector to obtain a recombinant DNA fragment which contains said DNA fragment and is capable of undergoing replication;

c) transforming a host cell with said recombinant DNA fragment to isolate a transformant which can express said polypeptide; and

d) culturing said transformant to allow the transformant to produce said polypeptide and recovering said polypeptide from resulting cultured mixture.

68. A polypeptide exhibiting bone stimulatory activity in mammals, the polypeptide being up to 65 amino acids in length and having the sequence identified as SEQ ID NO:11, SEQ ID NO:12, or SEQ ID NO:13; analogues thereof wherein the amino acids in the sequence may be substituted, deleted or added, so long as the bone stimulatory activity in mammals derived the three dimensional structure of the sequence is preserved; and conjugates of each of the polypeptides or analogues thereof, wherein if the polypeptide sequence contains a cysteine residue, there are at least two cysteine residues.

69. A polypeptide of any of claim 68 wherein the polypeptide is substantially pure and the amino acid sequence has a molecular weight in the range of from about 1000 to about 4000, or from about 1500 to about 3000, or from about 1500 to about 1800.

70. A first polypeptide comprising a sequence of amino acids up to 69 amino acids in length and sufficiently duplicative of a second polypeptide comprising an amino acid sequence corresponding an amino acid of claim 68 or 69, or a functionally equivalent homologue thereof, such that the first polypeptide is encoded by a DNA that hybridizes under stringent conditions with DNA encoding the second polypeptide.

71. A first polypeptide of claim 70, up to 60 amino acids in length.

72. A first polypeptide of claim 70, up to 50 amino acids in length.

73. A first polypeptide of claim 70, up to 40 amino acids in length.

74. A first polypeptide of claim 70, up to 30 amino acids in length.

75. A first polypeptide of claim 70, up to 20 amino acids in length wherein the amino acid sequence includes no cysteine residues or at least two cysteine residues.

76. A first polypeptide of claim 70, up to 14 amino acids in length.

77. A chimeric bone stimulating factor comprising an amino acid sequence of claim 68 or 69.

78. An agent for use in prevention and treatment of a bone reduction related disease which comprises a polypeptide of claim 68 or 69 as an active ingredient.

79. A pharmaceutical composition for promoting bone growth, comprising a therapeutically effective amount of a polypeptide of claim 68 or 69.

80. A method of increasing bone growth in a mammal by administering a therapeutically effective amount of a polypeptide having an amino acid sequence of a polypeptide defined in claim 68 or 69.

81. The use of a polypeptide of claim 68 or 69 for the treatment of osteoporosis.

82. The use of a polypeptide of claim 68 or 69 to promote bone growth in a mammal.

83. The use of a polypeptide having a sequence according to claim 68 or 69 in the preparation of a medicament for use in promoting bone growth or the treatment of osteoporosis.

84. A diagnostic kit for determining the presence of a polypeptide of claim 68 or 69 comprising an antibody to a said polypeptide linked to a reporter system wherein the reporter system produces a detectable response when a predetermined amount of the polypeptide and the antibody are bound together.

85. An antibody which binds to a polypeptide defined in claim 68 or 69, synthesized using the polypeptide.

86. An isolated DNA fragment which encodes the expression of any of the polypeptides of claim 68 or 69, and DNA which differs from the fragment due to the degeneracy of the genetic code.

87. A vector comprising a DNA sequence which encodes the expression of any of the polypeptides of claim 68 or 69.

88. A process for producing a polypeptide of claim 68 or 69, which comprises:

a) preparing a DNA fragment containing a nucleotide sequence which encodes said polypeptide;

b) incorporating said DNA fragment into an expression vector to obtain a recombinant DNA fragment which contains said DNA fragment and is capable of undergoing replication;

c) transforming a host cell with said recombinant DNA fragment to isolate a transformant which can express said polypeptide; and

d) culturing said transformant to allow the transformant to produce said polypeptide and recovering said polypeptide from resulting cultured mixture.

89. An isolated DNA sequence encoding the amino acid sequence set forth in any of claims 1 to 8, 32 to 37, or 56 or 57, or an analogue thereof, wherein the amino acids in the sequence may be substituted, deleted or added, so long as bone stimulatory activity in mammals derived from the three dimensional conformation of the sequence is preserved in a polypeptide comprising the amino acid sequence; sequences which hybridize to the DNA and encode an amino acid sequence of a polypeptide which displays bone stimulatory activity in mammals; and DNA which differs from the sequence due to the degeneracy of the genetic code.

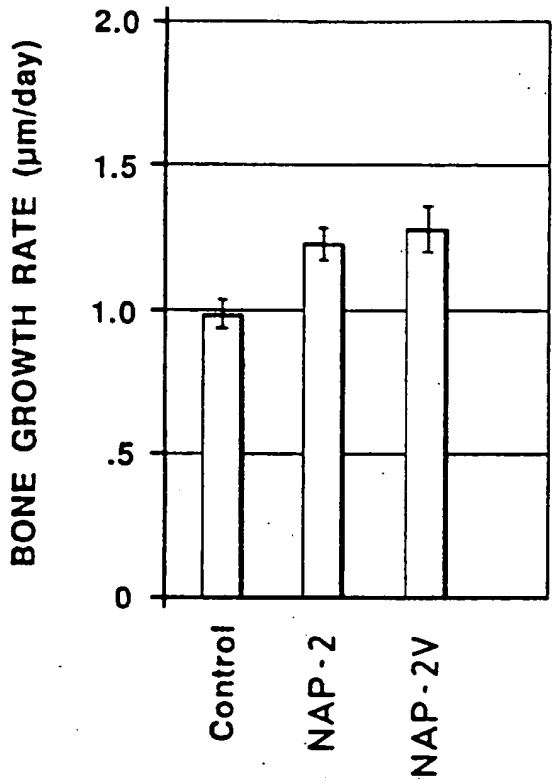

FIGURE 1

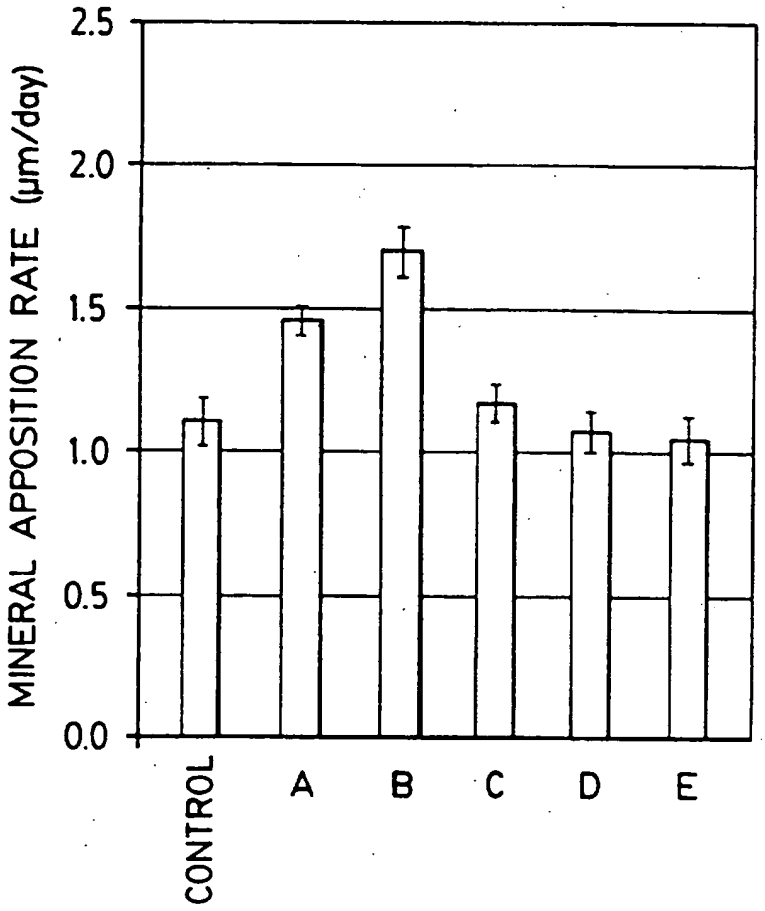
MINERAL APPOSITION RATE (µm/day)
2.5
2.0
1.5
1.0
0.5
0.0
CONTROL
A
B
C
D
E

FIGURE 2

Active Sequences:

SEQ ID NO:1 (MW=7670) AELRCMCIKTTSGIHPKNIQSLEVIGKGTHCNQVEVIATLKDGRKICLDPDAPRIKKIVQKKLAGDESAD

6 10 15 20 25 30 35 40 45 50 55 60 65 70

SEQ ID NO:2 (MW=8218) DSDLYAELRCMCIKTTSGIHPKNIQSLEVIGKGTHCNQVEVIATLKDGRKICLDPDAPRIKKIVQKKLAGDESAD

SEQ ID NO:11 (MW=2850) DSDLYAELRCMCIKTTSGIHPKNIQS

SEQ ID NO:12 (MW=1530) IKTTSGIHPKNIES

SEQ ID NO:13 (MW=1862) CMCIKTTSGIHPKNIQS

Inactive Sequences:

SEQ ID NO:14 (MW=1750) MCIKTTSGIHPKNIQS

SEQ ID NO:15 (MW=1643) CIKTTSGIHPKNIQS

FIGURE 3

INTERNATIONAL SEARCH REPORT

Interna[illegible]plication No
PCT/CA 96/00653

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/10 C07K14/52 A61K38/19 G01N33/53 C07K16/24
C12N15/63

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 March 1997	17.03.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Montero Lopez, B

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 11, 17 March 1995, MD US, pages 6338-6344, XP002022825 JAN E. EHLERT ET AL.: "Limited and defined truncation at the C terminus enhances receptor binding and degranulation activity of the neutrophil-activating peptide 2 (NAP-2)" cited in the application see abstract see page 6338, right-hand column, paragraph 2 - page 6339, left-hand column, paragraph 1 see page 6340, right-hand column, paragraph 2 - page 6342, left-hand column, paragraph 1 see page 6343, left-hand column, paragraph 1 - page 6344, left-hand column, paragraph 2 ---	1-24, 33-55, 64-76, 85-89
A	WO 94 05309 A (UNIVERSITY OF LOUISVILLE RESEARCH FOUNDATION) 17 March 1994 see page 2, line 1 - page 3, line 8 & US 5 304 542 A cited in the application ---	25-31, 56-62, 77-83
P,X	WO 95 28172 A (OSTEOPHARM LIMITED ;TAM CHERK SHING (CA)) 26 October 1995 see page 4, line 25 - page 7, line 9 see page 14, line 5 - page 16, line 20 see page 17, line 4 - line 34 -----	1-89

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 96/00653

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 28-30,59-61,80-82
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although these claims are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compounds.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter[illegible]plication No
PCT/[illegible]96/00653

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9405309 A	17-03-94	US 5304542 A	19-04-94
		AU 5092093 A	29-03-94
WO 9528172 A	26-10-95	US 5578569 A	26-11-96
		AU 2211195 A	10-11-95
		CA 2188172 A	26-10-95
		EP 0756490 A	05-02-97